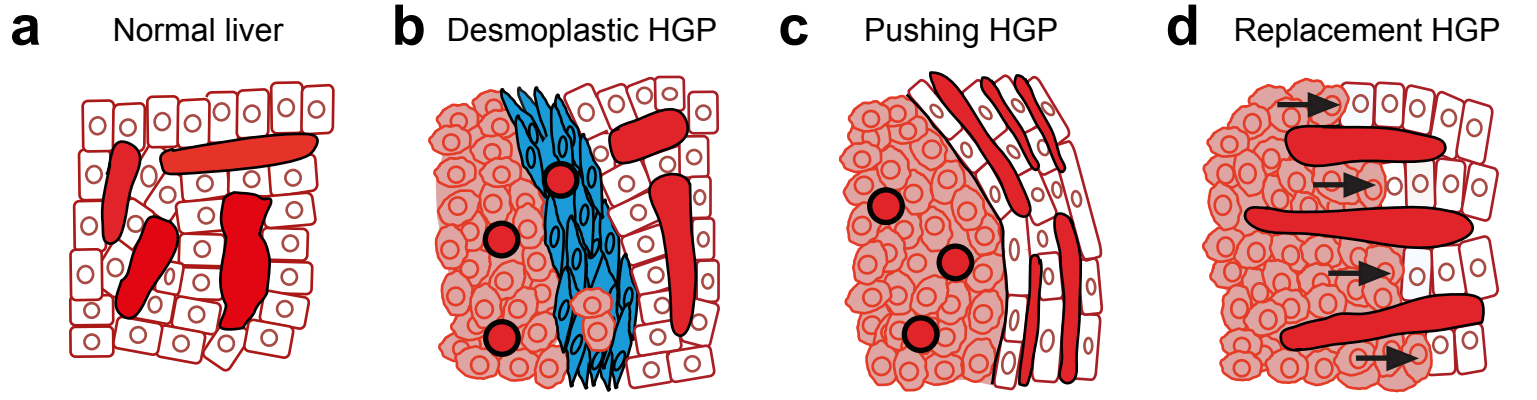
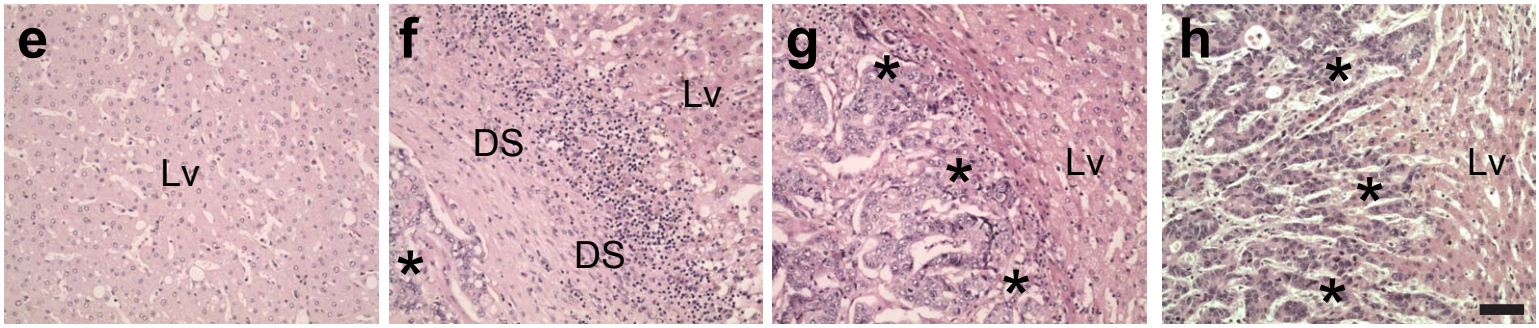
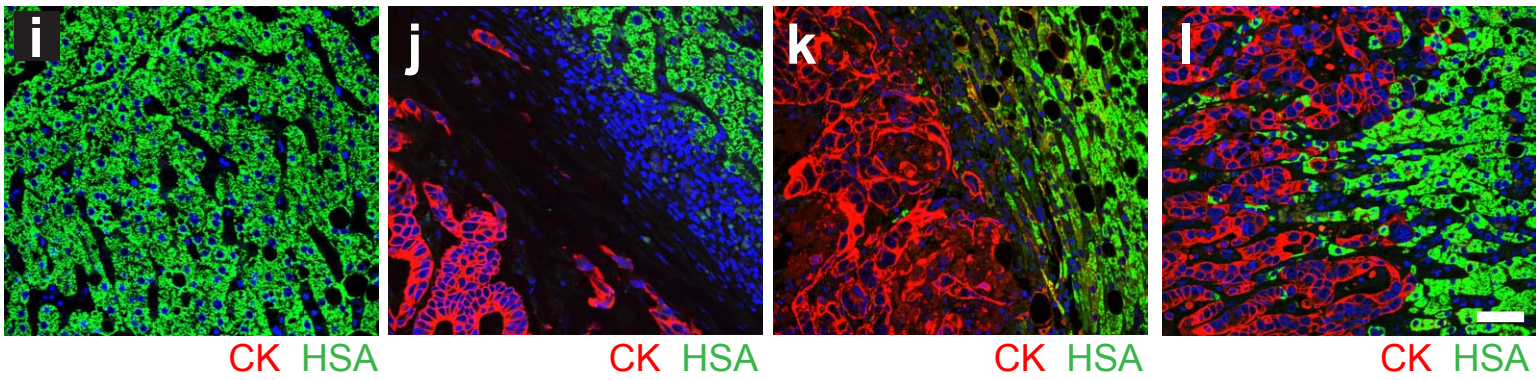
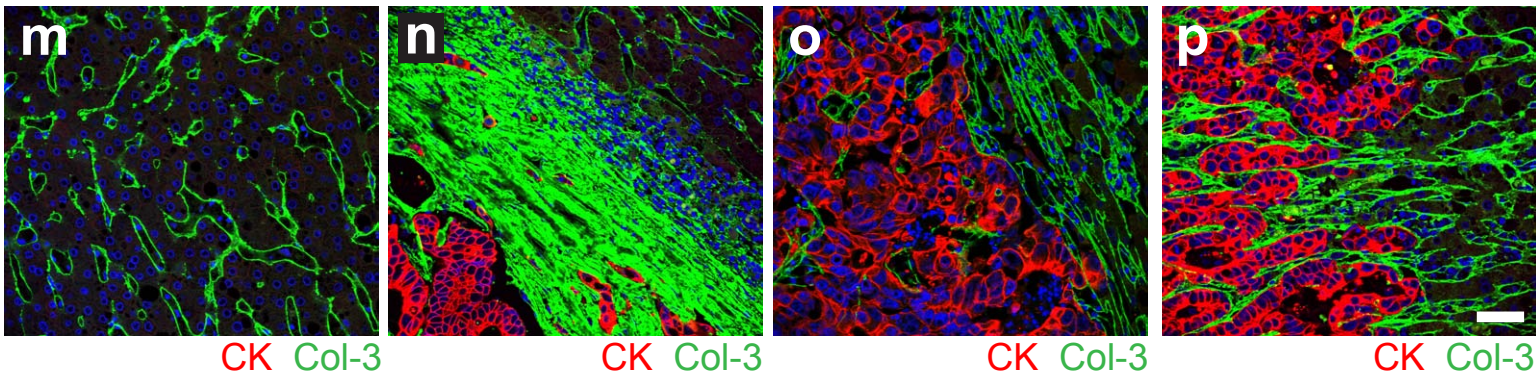
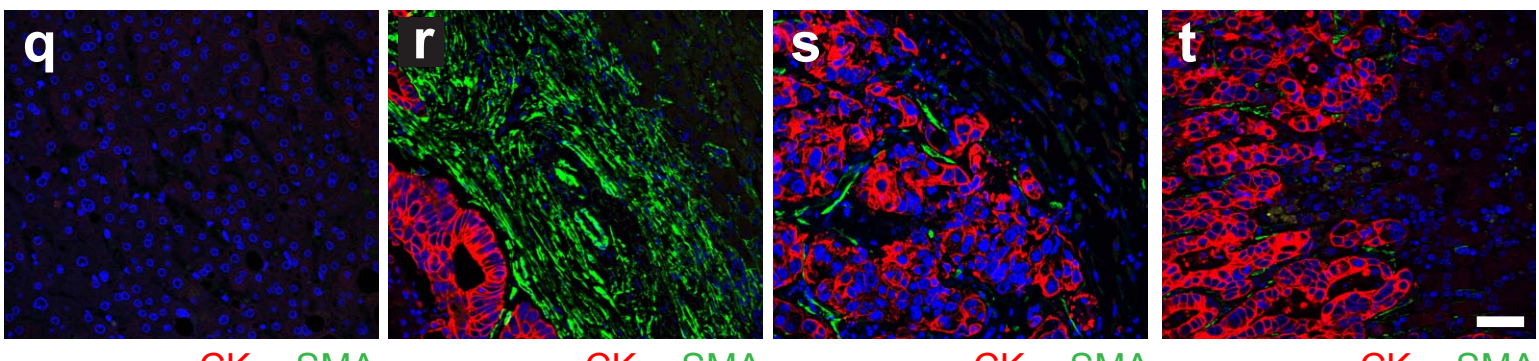
Supplementary Figure 1











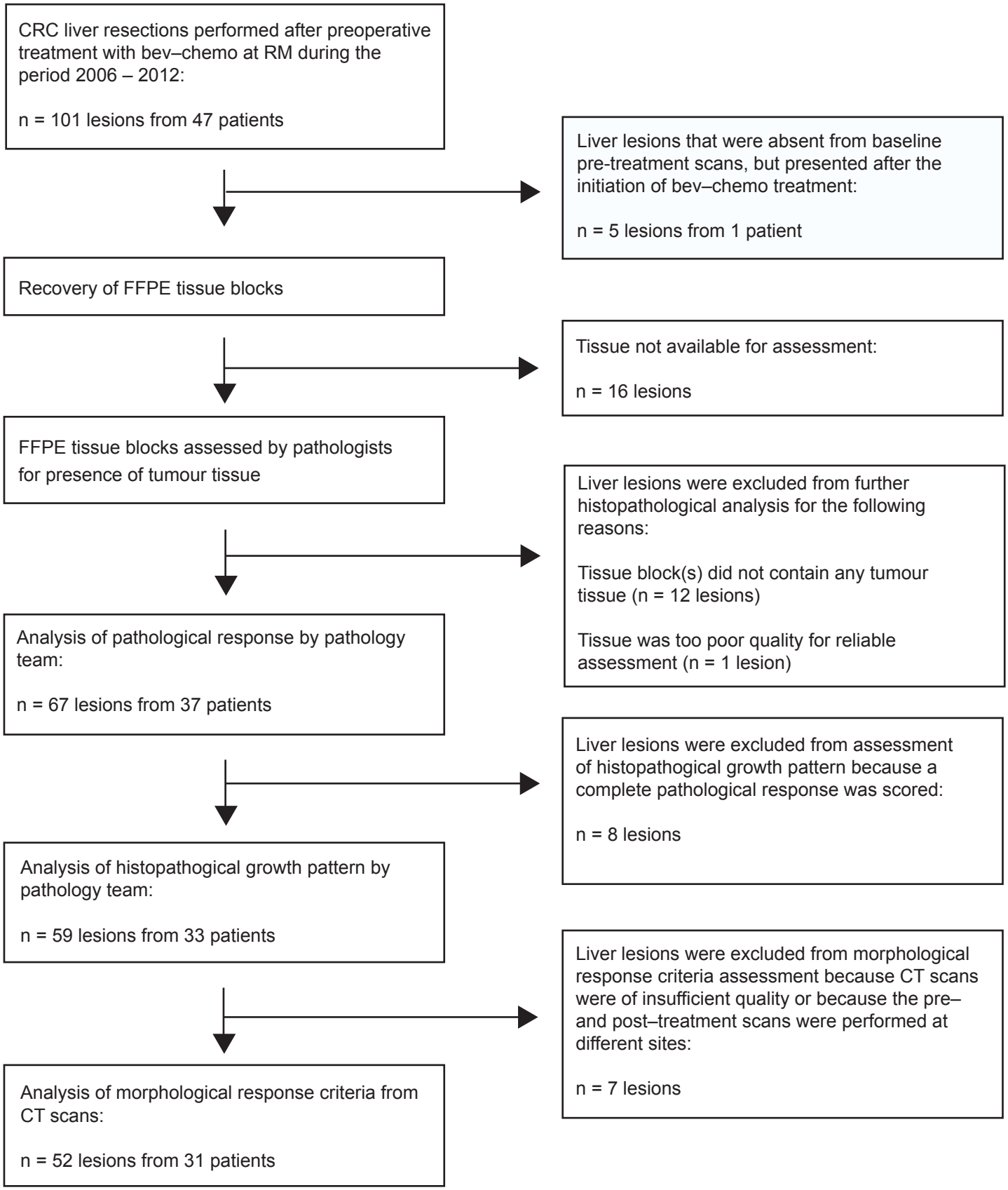
See overleaf for figure legend

Supplementary Figure 1 Morphology of the three histopathological growth patterns (HGPs) of colorectal cancer liver metastases

a–h. Diagrams and H&E–stainings illustrate the morphology of normal liver or the morphology of the tumor–normal liver interface in human CRC liver metastases with a desmoplastic, pushing or replacement HGP.

i–t. To confirm the distinct tumor–stroma interaction that occurs in each HGP, we performed additional staining for hepatocyte specific antigen (HSA), collagen–3 (col–3) and alpha smooth muscle actin (αSMA). In **normal liver**, HSA labeled hepatocytes (**i**), col–3 labeled sinusoidal blood vessels (**m**), whilst αSMA labeled neither hepatocytes nor sinusoidal blood vessels (**q**). In the **desmoplastic HGP**, a desmoplastic stroma physically separates cancer cells from normal liver (**b,f**). Co–staining for pan–cytokeratin (CK) to detect cancer cells and HSA to detect hepatocytes confirmed physical separation of cancer cells and normal liver (**j**), whilst co–staining for pan–cytokeratin and col–3, or pan–cytokeratin and αSMA, confirmed the presence of a desmoplastic stroma abundant in collagen (**n**) and αSMA–positive fibroblasts (**r**), respectively. In the **pushing HGP**, cancer cells and normal liver are in close contact with no intervening desmoplastic stroma (**c,g**) which was confirmed by co–staining for CK and HSA (**k**) or CK and αSMA (**s**). Another feature of the pushing HGP, physical compression of sinusoidal vessels in adjacent normal liver tissue, was confirmed by co–staining for pan–cytokeratin and col–3 (**o**). In the **replacement HGP**, cancer cells infiltrate the liver parenchyma and replace hepatocytes without disturbing the vascular architecture of the liver; no desmoplastic stroma is observed (**d,h**). Supporting this, co–staining for CK and HSA confirmed the invasion of cancer cells into liver parenchyma (**l**). Co–staining for CK and col–3 showed that the vascular architecture of the adjacent liver was preserved at the tumor–liver interface (**p**). Lack of αSMA staining confirmed the absence of a desmoplastic stroma (**t**). Asterisk, cancer cells. DS, desmoplastic stroma. Lv, normal liver. Scale bars, 50 μM.

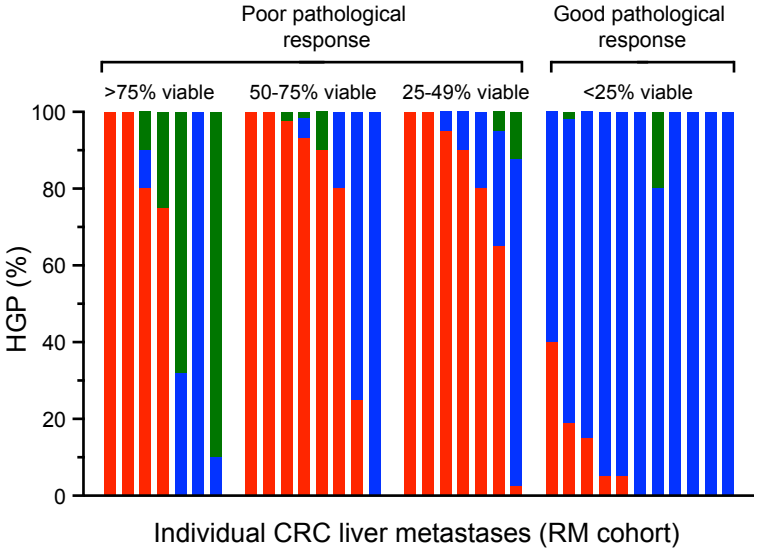
Supplementary Figure 2



Supplementary Figure 2 Consort diagram for RM cohort

Consort diagram to illustrate how cases of CRC liver metastases from patients treated preoperatively with bev–chemo at RM were selected for inclusion in the study or excluded.

Supplementary Figure 3



		Response		
		Poor	Good	Total
No. of lesions	<50% R	6 (35.3%)	11 (64.7%)	17 (100%)
	≥50% R	16 (100%)	0 (0%)	16 (100%)
	Total	22 (66.7%)	11 (33.3%)	33 (100%)

$P < 0.001$

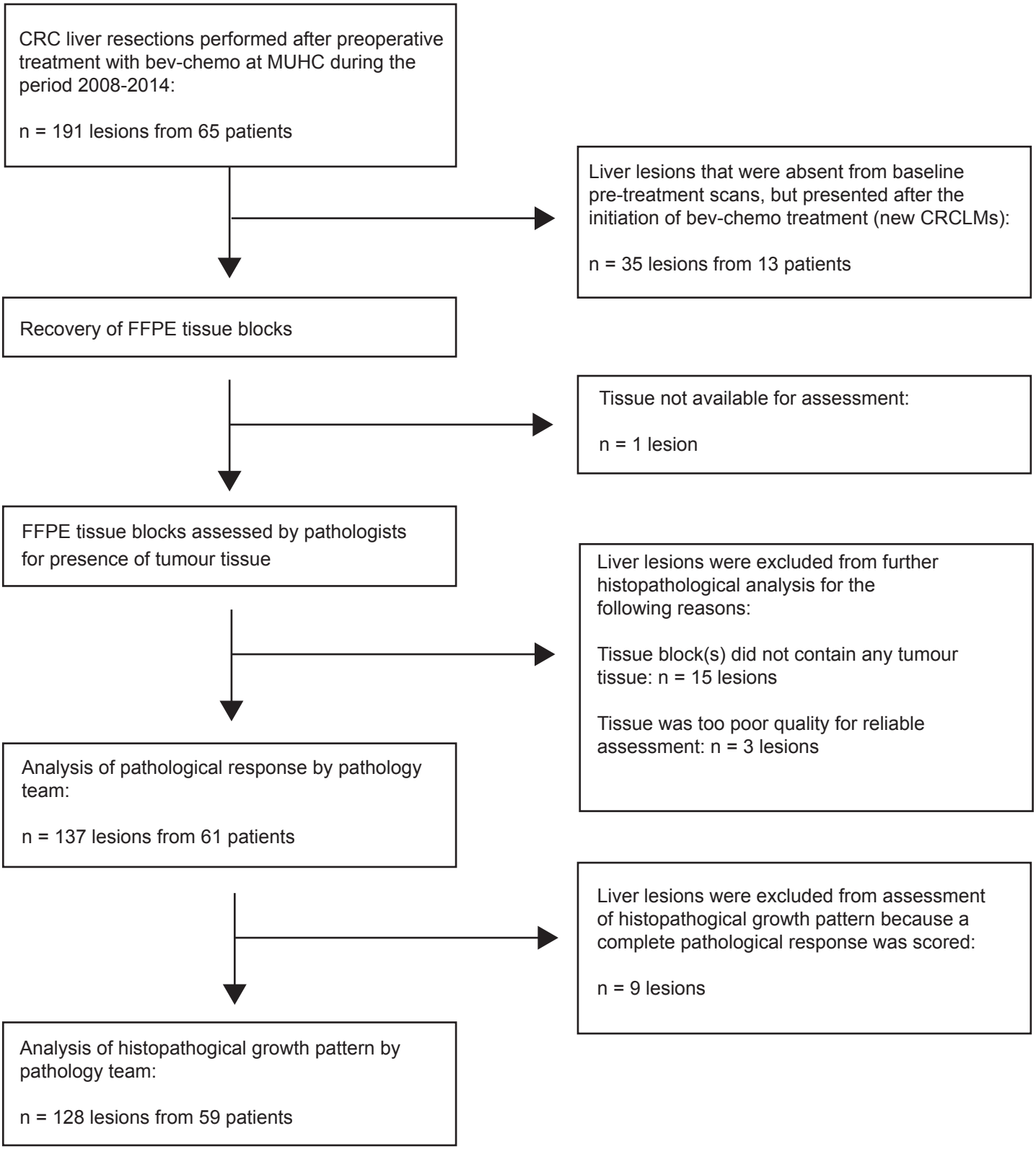
		Response		
		Poor	Good	Total
No. of lesions	<50% D	18 (100%)	0 (0%)	18 (100%)
	≥50% D	4 (26.7%)	11 (73.3%)	15 (100%)
	Total	22 (66.7%)	11 (33.3%)	33 (100%)

$P < 0.001$

Supplementary Figure 3 Correlation between HGP and pathological response in an analysis restricted to one lesion per patient (RM cohort)

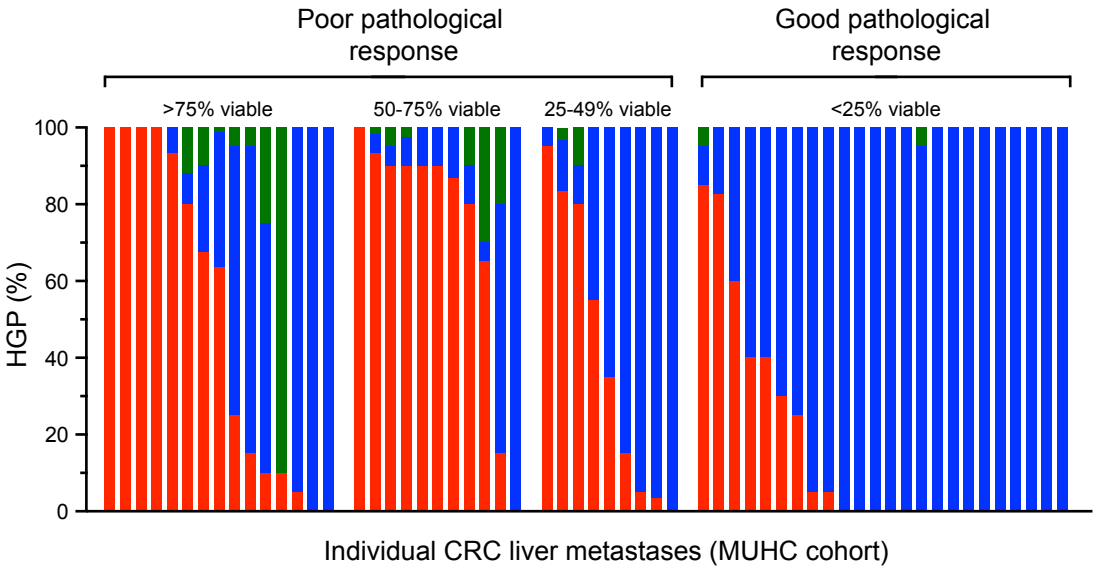
Data are presented from the same series of 33 patients as depicted in Figure 1b, but for this analysis only one lesion per patient was used. The graph shows the % HGP (replacement, desmoplastic, pushing) scored in the largest lesion from each patient. Lesions scored as >75%, 50-75% or 25-49% viable were considered to be poor responders, whilst lesions scored as <25% viable were considered good responders. Lesions with a substantial (≥50%) replacement HGP were significantly enriched in the poor responder group when compared with good responders ($P < 0.001$), whilst lesions with a substantial (≥50%) desmoplastic HGP were significantly enriched in the good responder group when compared with poor responders ($P < 0.001$). The χ^2 test was used to determine statistical significance (see 2x2 contingency tables).

Supplementary Figure 4



Supplementary Figure 4 Consort diagram for MUHC cohort
Consort diagram to illustrate how cases of CRC liver metastases from patients treated preoperatively with bev-chemo at MUHC were selected for inclusion in the study or excluded.

Supplementary Figure 5



		Response		
		Poor	Good	Total
No. of lesions	<50% R	14 (40%)	21 (60%)	35 (100%)
	≥50% R	21 (87.5%)	3 (12.5%)	24 (100%)
	Total	35 (59.3%)	24 (40.7%)	59 (100%)

$P < 0.001$

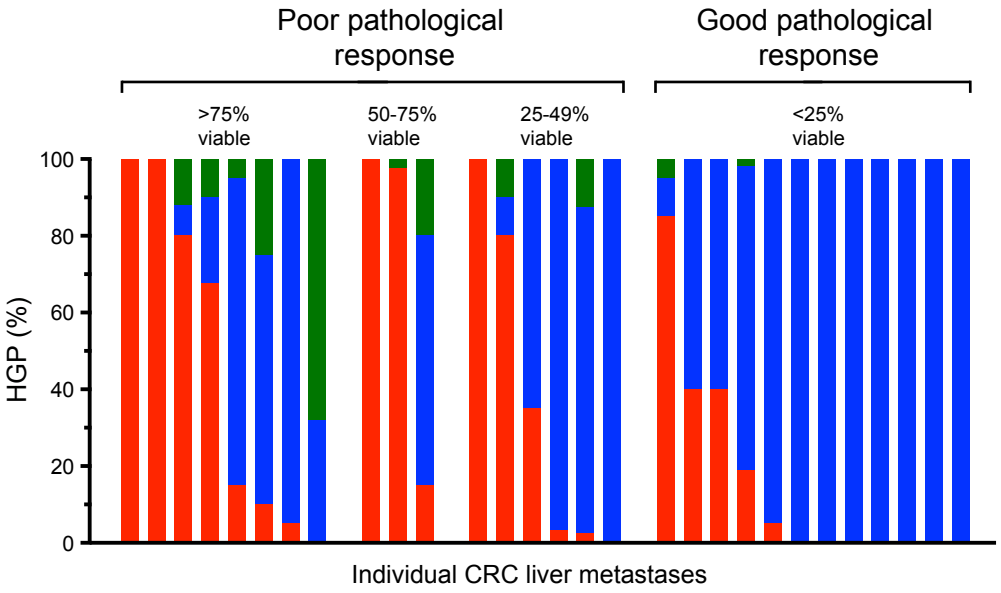
		Response		
		Poor	Good	Total
No. of lesions	<50% D	14 (40%)	21 (60%)	35 (100%)
	≥50% D	21 (87.5%)	3 (12.5%)	24 (100%)
	Total	35 (59.3%)	24 (40.7%)	59 (100%)

$P < 0.001$

Supplementary Figure 5 Correlation between HGP and pathological response in an analysis restricted to one lesion per patient (MUHC cohort)

Data are presented from the same series of 59 patients as depicted in Figure 1f, but for this analysis only one lesion per patient was used. The graph shows the % HGP (replacement, desmoplastic, pushing) scored in the largest lesion from each patient. Lesions scored as >75%, 50-75% or 25-49% viable were considered to be poor responders, whilst lesions scored as <25% viable were considered good responders. Lesions with a substantial (≥50%) replacement HGP were significantly enriched in the poor responder group when compared with good responders ($P < 0.001$), whilst lesions with a substantial (≥50%) desmoplastic HGP were significantly enriched in the good responder group when compared with poor responders ($P < 0.001$). The χ^2 test was used to determine statistical significance (see 2x2 contingency tables).

Supplementary Figure 6



		Response		
		Poor	Good	Total
No. of lesions	<50% R	9 (45%)	11 (55%)	20 (100%)
	≥50% R	8 (88.9%)	1 (11.1%)	9 (100%)
	Total	17 (58.6%)	12 (41.4%)	29 (100%)

P = 0.0264

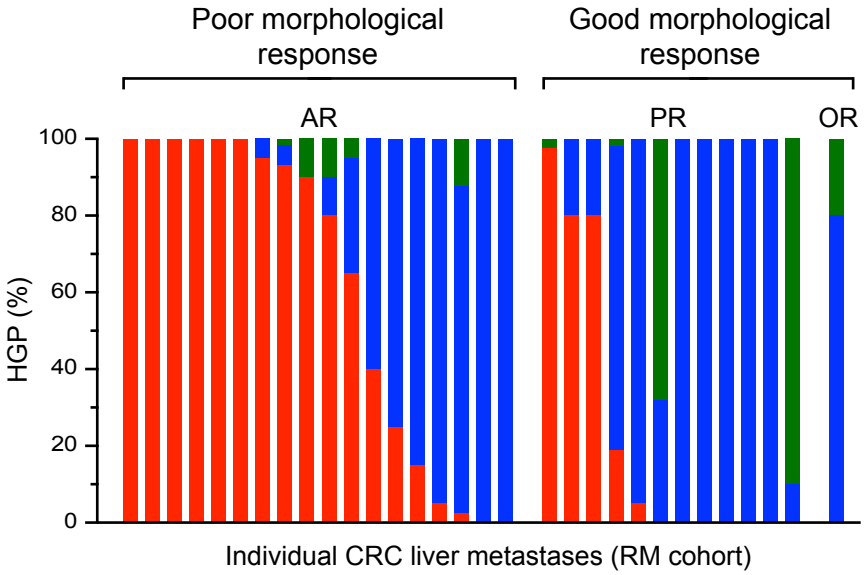
		Response		
		Poor	Good	Total
No. of lesions	<50% D	9 (90%)	1 (10%)	10 (100%)
	≥50% D	8 (42.1%)	11 (57.8%)	19 (100%)
	Total	17 (58.6%)	12 (41.4%)	29 (100%)

P = 0.0128

Supplementary Figure 6 The HGPs correlate with pathological response in patients presenting with a single lesion only

The graph shows the HGPs and pathological response in 29 patients that presented with a single lesion only. Graph shows the % HGP (replacement, desmoplastic, pushing) scored in each lesion from each patient. Lesions scored as >75%, 50-75% or 25-49% viable were considered to be poor responders, whilst lesions scored as <25% viable were considered good responders. Lesions with a substantial (≥50%) replacement HGP were significantly enriched in the poor responder group when compared with good responders (*P*=0.0264). Lesions with a substantial (≥50%) desmoplastic HGP were significantly enriched in the good responder group when compared with poor responders (*P*=0.0128). The χ^2 test was used to determine statistical significance (see 2x2 contingency table).

Supplementary Figure 7



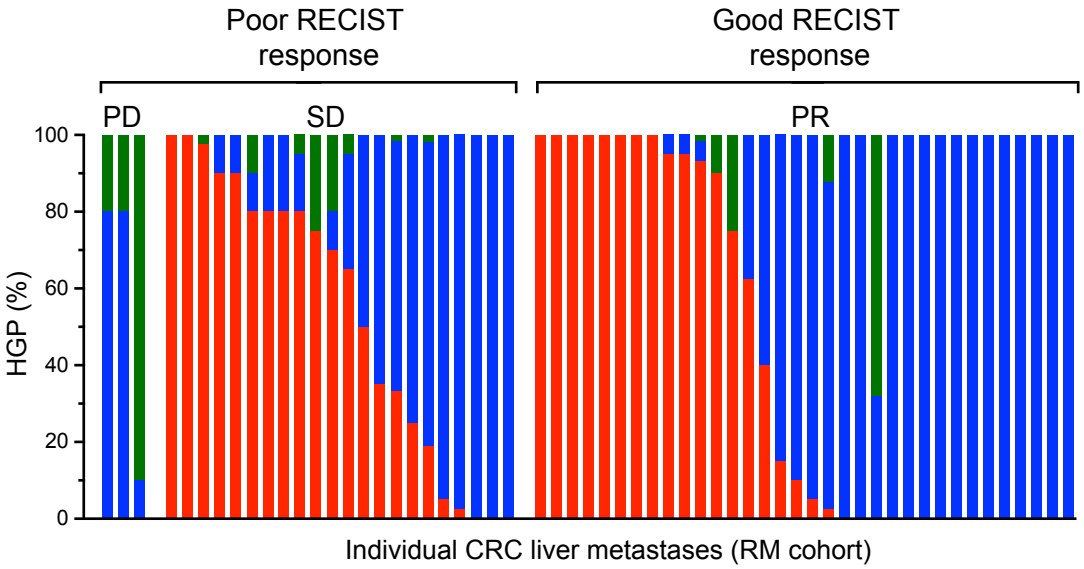
		Response		
		Poor	Good	Total
No. of lesions	<50% R	7 (41.2%)	10 (58.8%)	17 (100%)
	≥50% R	11 (78.6%)	3 (21.4%)	14 (100%)
	Total	18 (58.1%)	13 (41.9%)	31 (100%)

P = 0.0357

Supplementary Figure 7 Correlation between HGP and morphological response in an analysis restricted to one lesion per patient (RM cohort)

Data are presented from the same series of 31 patients as depicted in Figure 2g, but for this analysis only one lesion per patient was used. The graph shows the % HGP (replacement, desmoplastic, pushing) scored in the largest lesion from each patient. Lesions scored as having an absent morphological response (AR) were considered to be poor responders, whilst those undergoing a partial (PR) or optimal (OR) morphological response were considered to be good responders. Lesions with ≥50% replacement HGP were significantly enriched in poor responders compared to good responders (P = 0.0357). The χ^2 test was used to determine statistical significance (see 2x2 contingency table).

Supplementary Figure 8



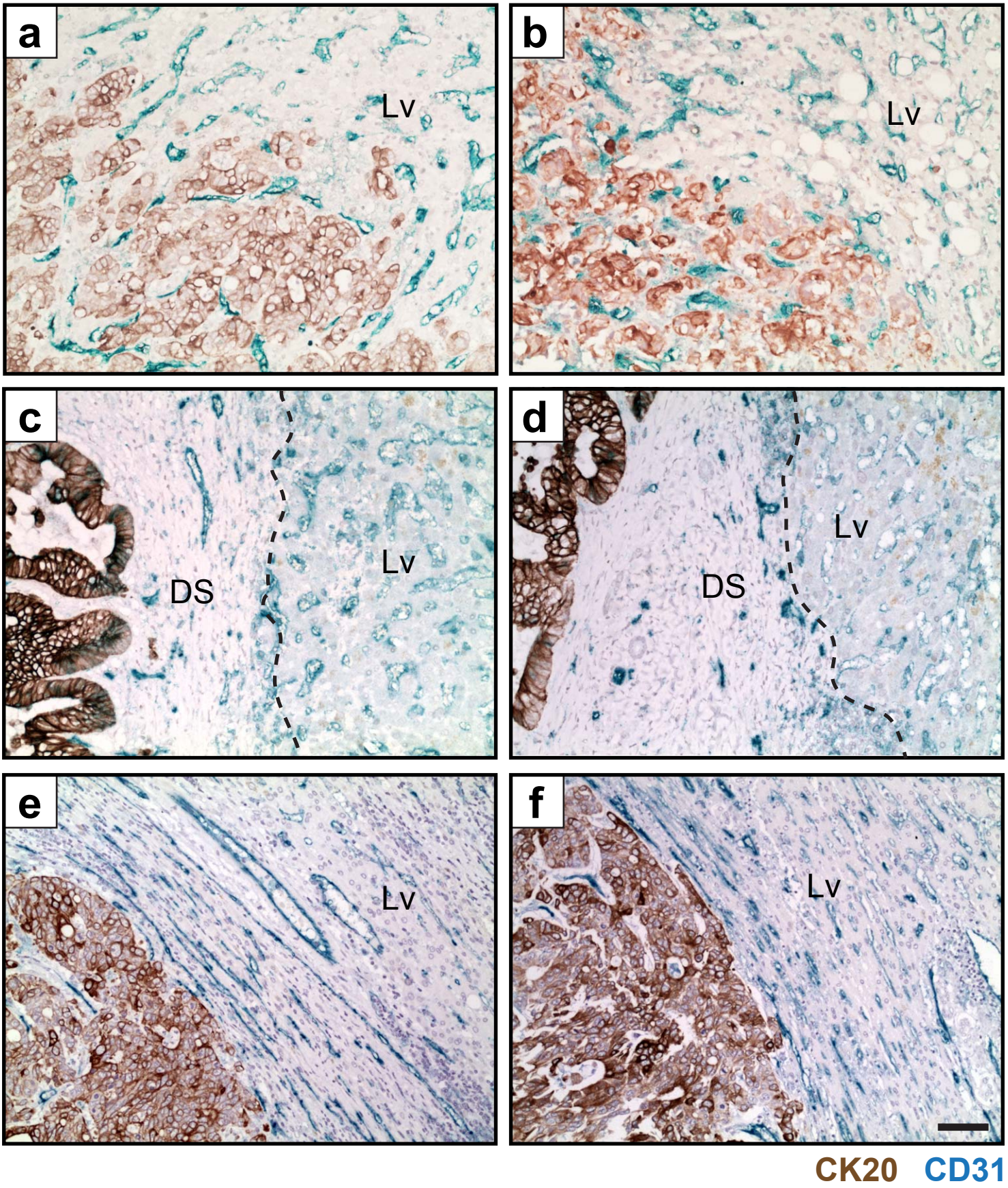
		Response		
		Poor	Good	Total
No. of lesions	<50% R	12 (37.5%)	20 (62.5%)	32 (100%)
	≥50% R	13 (48.1%)	14 (51.9%)	27 (100%)
	Total	25 (42.4%)	34 (57.6%)	59 (100%)

P = 0.440

Supplementary Figure 8 The HGPs do not correlate with response when using RECIST criteria as a response measure

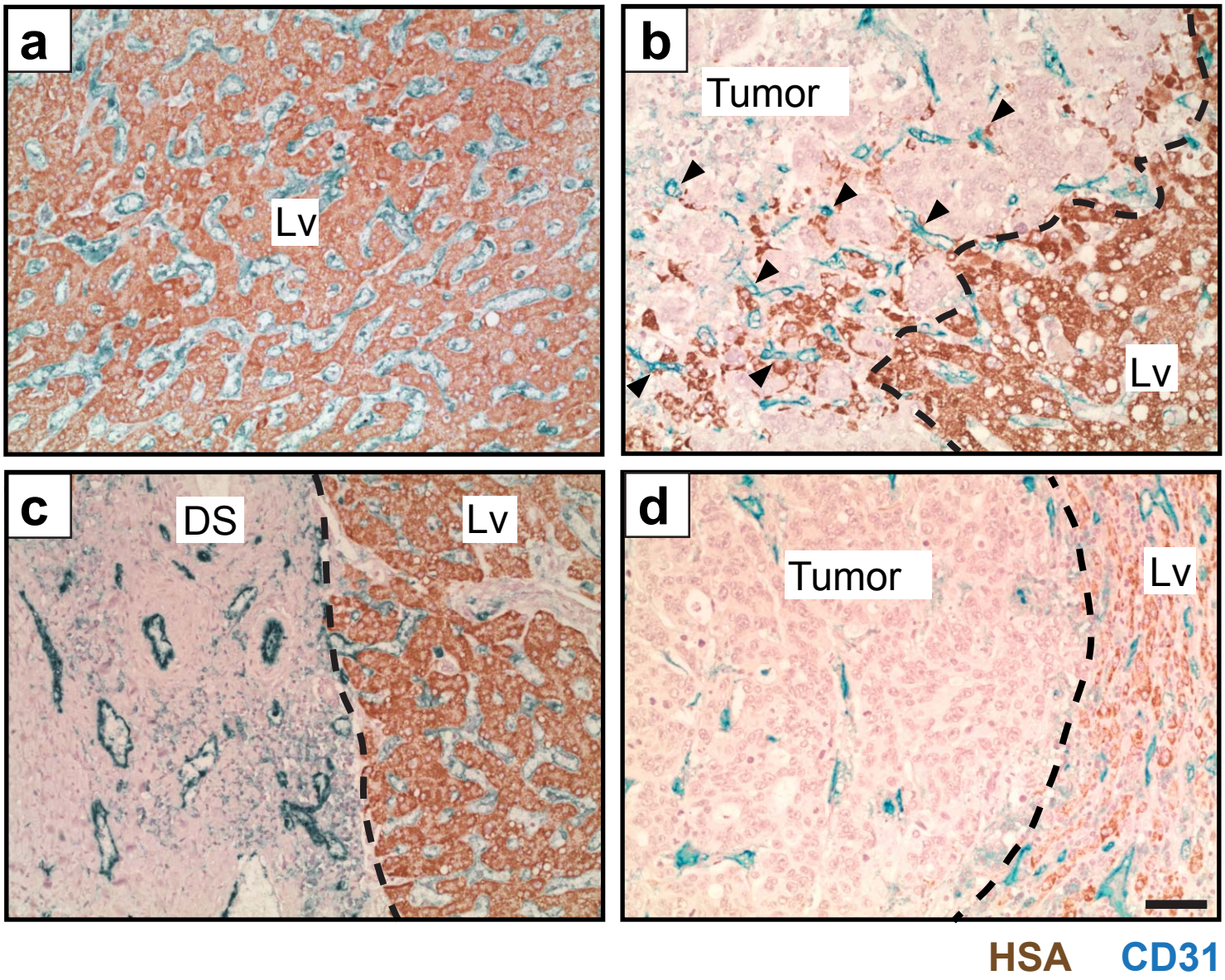
Response to bev-chemo was scored using RECIST criteria in order to categorise individual lesions as: progressive disease (PD), stable disease (SD) or partial response (PR). Graph shows the % HGP scored in each individual lesion (replacement, desmoplastic, pushing) with lesions grouped according to response: PD, SD or PR (n = 59 liver metastases from 33 patients). Lesions scored as PD or SD were considered to be poor responders, whilst lesions scored as PR were considered to be good responders. Lesions with a substantial (≥50%) replacement HGP were not significantly enriched in the poor responder group when compared with good responders (*P*=0.440). The χ^2 test was used to determine statistical significance (see 2x2 contingency table).

Supplementary Figure 9



Supplementary Figure 9 Staining for blood vessels in the different histopathological growth patterns
Resection specimens of CRCLMs corresponding to the three different HGPs were stained for cytokeratin 20 (CK20) to identify cancer cells (brown) and CD31 to identify vessels (blue). **a,b.** Replacement HGP. Co-option of sinusoidal vessels by invading cancer cells is observed. **c,d.** Desmoplastic HGP. Co-option of sinusoidal vessels by cancer cells is physically precluded by the desmoplastic stroma (DS) that separates cancer cells from the normal liver (Lv). Dashed line indicates where the desmoplastic rim of the tumor meets the normal liver. **e,f.** Pushing HGP. Sinusoidal vessels that are present in the normal liver adjacent to the tumor are compressed, highly elongated and run in parallel with the tumor-liver interface, a topology that physically precludes the co-option of these vessels by invading cancer cells. DS, desmoplastic stroma. Lv, normal liver. Scale bar, 50 μM.

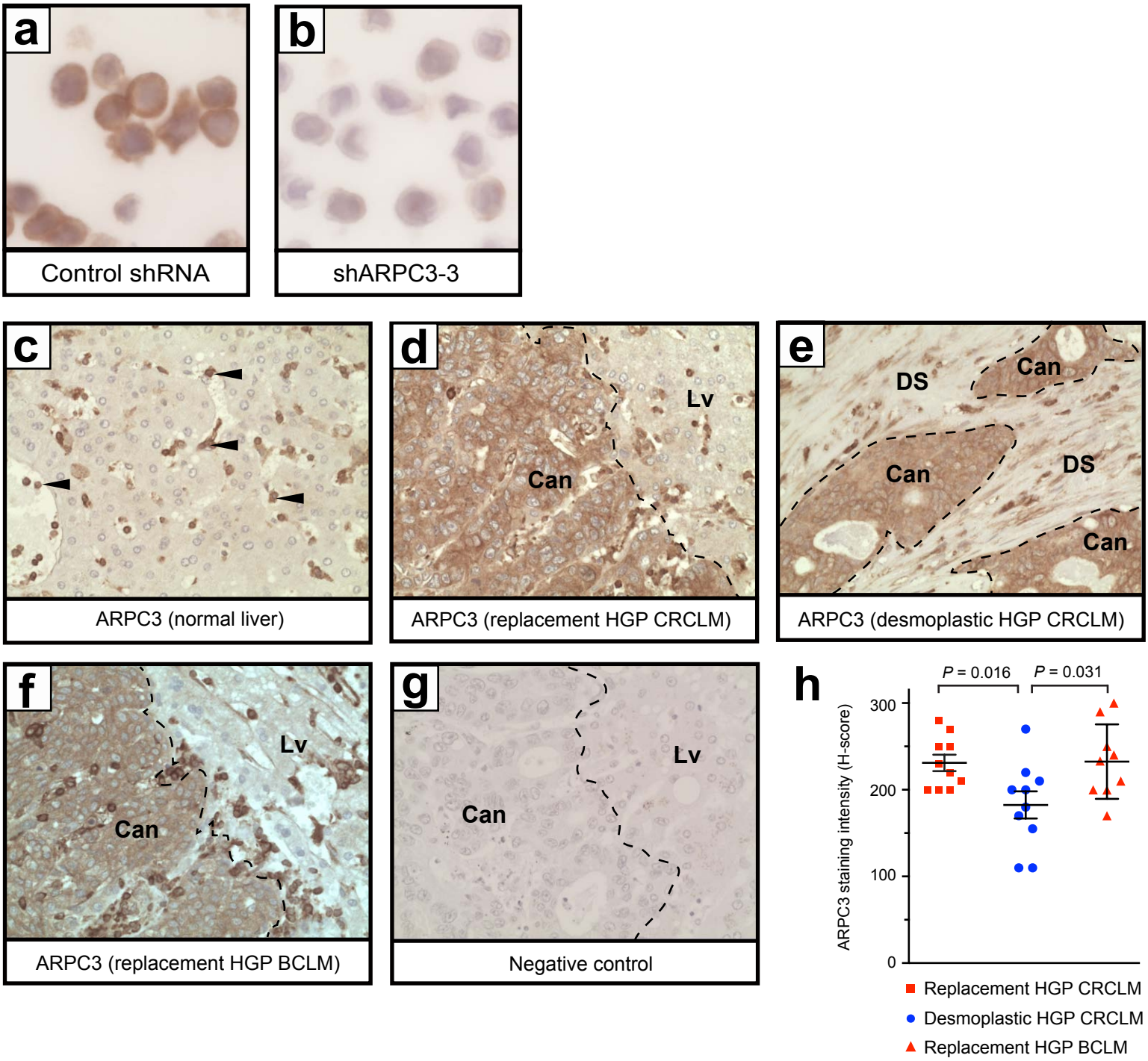
Supplementary Figure 10



Supplementary Figure 10 Co-staining for blood vessels and hepatocytes in the different histopathological growth patterns

Resection specimens of CRCLMs were stained for HSA to identify hepatocytes (brown) and CD31 to identify vessels (blue). **a.** Normal liver, **b.** replacement HGP, **c.** desmoplastic HGP, and **d**. pushing HGP. Dashed line indicates the interface where the tumor meets the normal liver. Arrowheads indicate co-opted sinsuoidal vessels that are still associated with hepatocytes. DS, desmoplastic stroma. Lv, normal liver. Scale bar, 50 μM.

Supplementary Figure 11



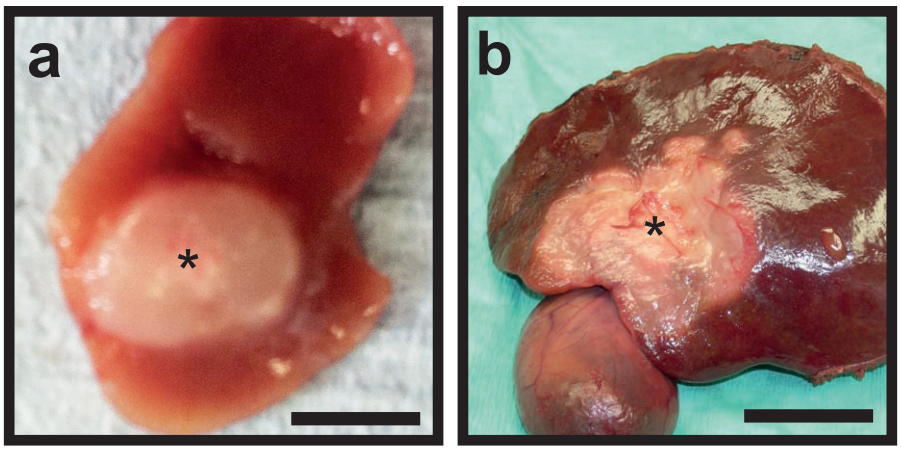
Supplementary Figure 11 Expression of the Arp2/3 subunit ARPC3 in human liver metastases

a,b. Validation of anti-ARPC3 antibody staining specificity
HT29 cells stably transfected with a control non-targeting shRNA (control shRNA) (**a**) or an ARPC3-targeted shRNA (shARPC3-3) (**b**) were prepared for FFPE sections and then stained using an anti-ARPC3 antibody (MABT95, Millipore). Loss of antigenicity in the knockdown cells (**b**) compared to the control cells (**a**) indicates that this antibody is specific for ARPC3.

c-e. Examples of ARPC3 staining in human liver metastasis specimens
Samples of human liver metastasis were stained using the anti-ARPC3 antibody. **c.** ARPC3 staining in normal liver. ARPC3 staining is limited to Kuppfer cells and immune cells within the lumen of vessels (arrowheads) and staining is absent / weak in hepatocytes. **d-f.** ARPC3 staining in cancer cells (Can) of a replacement HGP CRCLM (**d**), a desmoplastic HGP CRCLM (**e**) and a replacement HGP breast cancer liver metastasis (BCLM) (**f**). Panel **g** shows a negative control, where the same staining protocol was performed but the primary antibody was omitted. Can, cancer cells. Lv, normal liver parenchyma. DS, desmoplastic stroma.

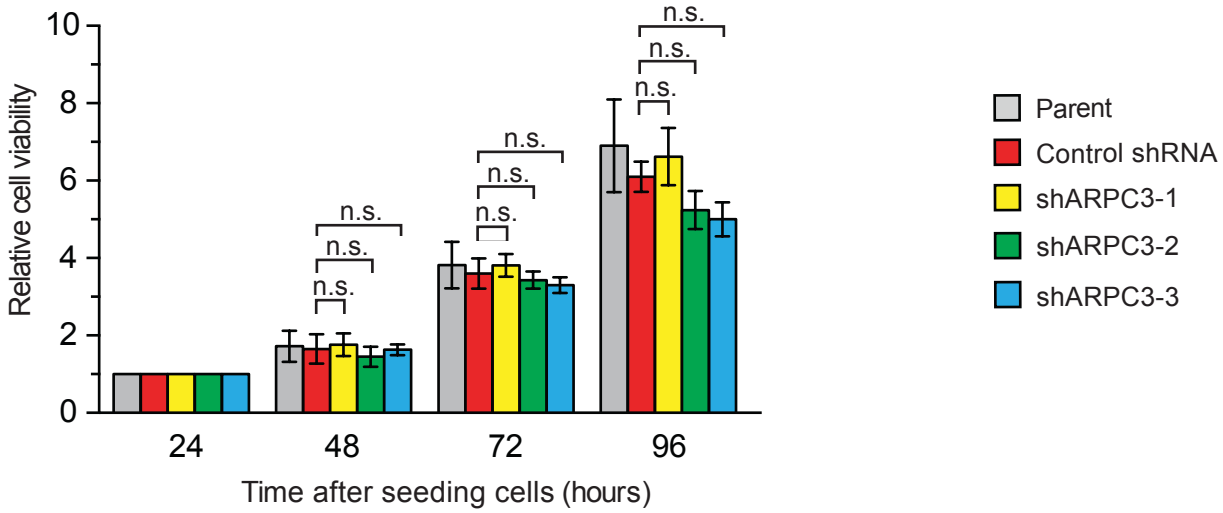
h. Quantification of ARPC3 staining in human liver metastasis specimens
The intensity of ARPC3 staining was scored in replacement HGP CRCLMs (n = 10), desmoplastic HGP CRCLMs (n = 10) and replacement HGP BCLMs (n = 9). Each data point on the graph is the intensity (H-score) for an individual

Supplementary Figure 12



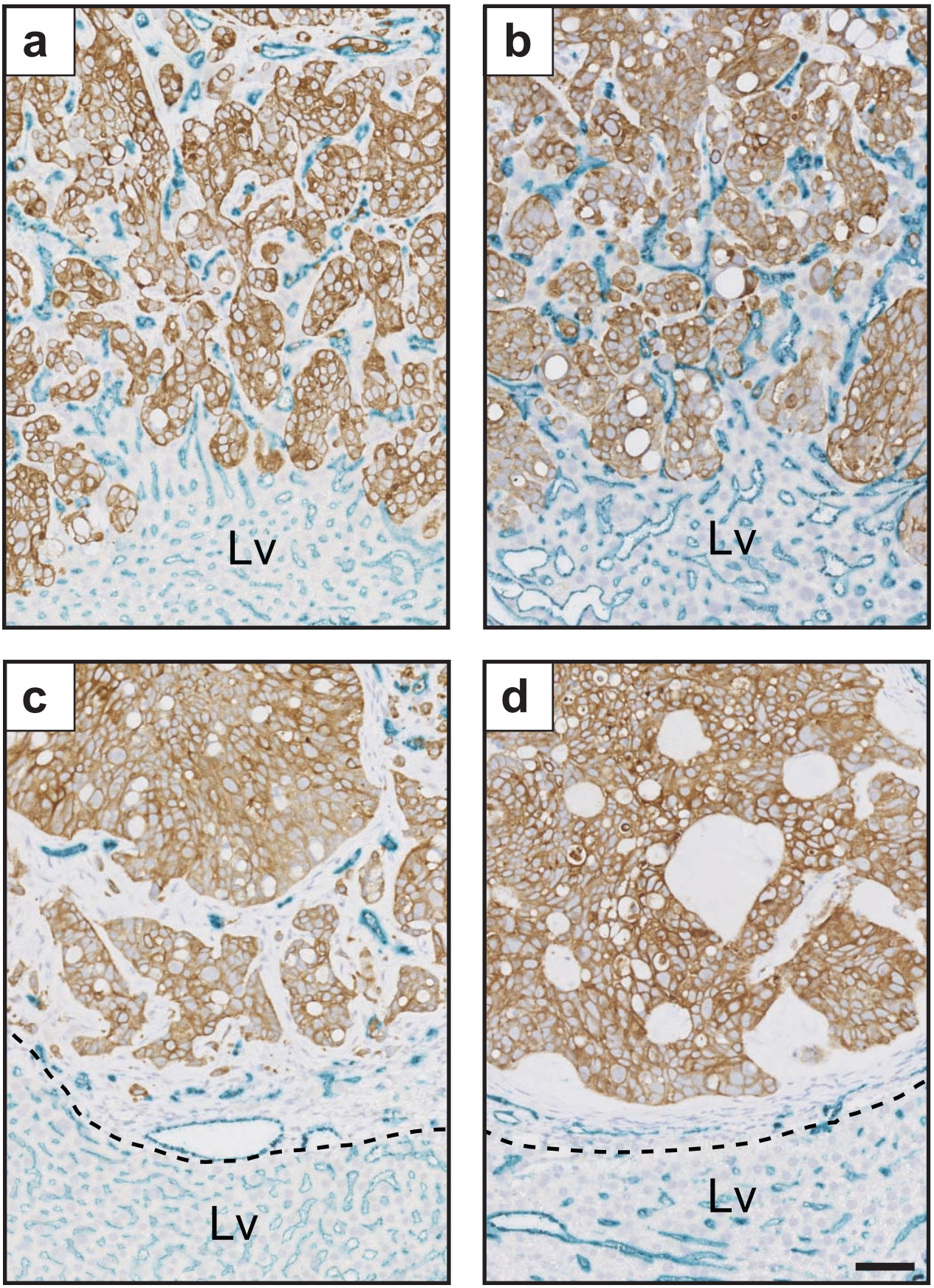
Supplementary Figure S12 Preclinical model of advanced liver metastasis
a. Macroscopic appearance of tumor formation in the left main lobe of the mouse liver after injection of HT29 cells. **b.** Macroscopic appearance of a human CRC liver metastasis resected from a patient (picture is courtesy of Mr Ali Majeed). Scale bar, 5 mm (**a**) or 5 cm (**b**). Tumor is indicated by an asterisk.

Supplementary Figure 13



Supplementary Figure 13 Knockdown of ARPC3 in HT29 cells does not alter cell proliferation
Proliferation of parental HT29 cells (Parent) and HT29 cells stably transduced with control shRNA, shARPC3-1, shARPC3-2 or shARPC3-3. The quantity of viable cells is expressed relative to the quantity measured at 24 hours ± SEM (n = 3 independent experiments). n.s., no significant difference (Student's t-test).

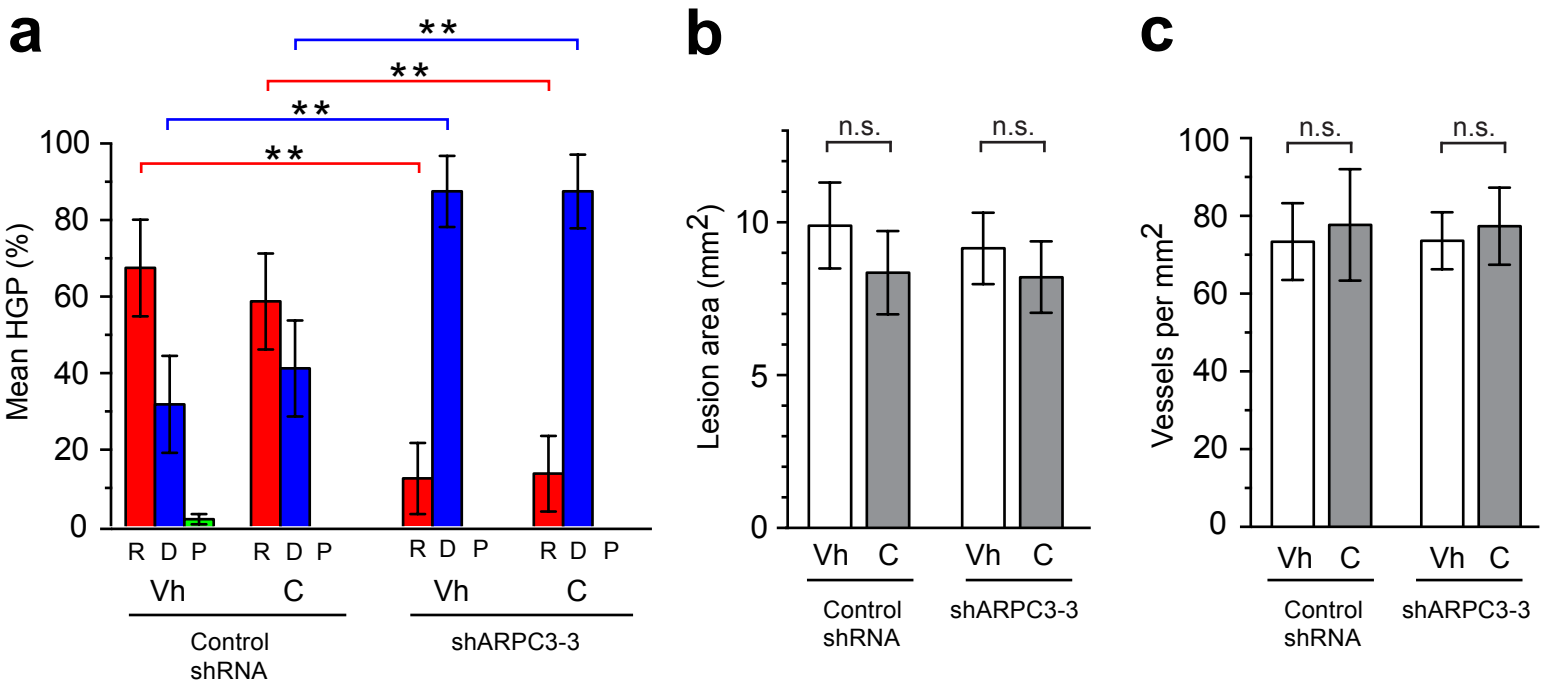
Supplementary Figure 14



Supplementary Figure 14 Staining for CD31 in HT29 tumours treated with B20-4.1.1 and capecitabine *in vivo*

a-d. HT29 tumors with normal ARPC3 levels (Control shRNA) or ARPC3 knockdown (shARPC3-3) were established in the livers of mice and treated with B20-4.1.1 plus capecitabine (BC) or vehicle (Vh) alone. Liver specimens harvested after two weeks of treatment were stained for CK20 to label tumor cells and CD31 to label blood vessels. Representative images of the tumour-liver interface are shown for Control shRNA tumors treated with Vh (**a**) or B/C (**b**) and for ARPC3 knockdown tumors treated with Vh (**c**) or BC (**d**). Dashed line in panels **c** and **d** indicates where the desmoplastic rim of the tumor meets the normal liver. Lv, normal liver. Scale bar, 60 μM.

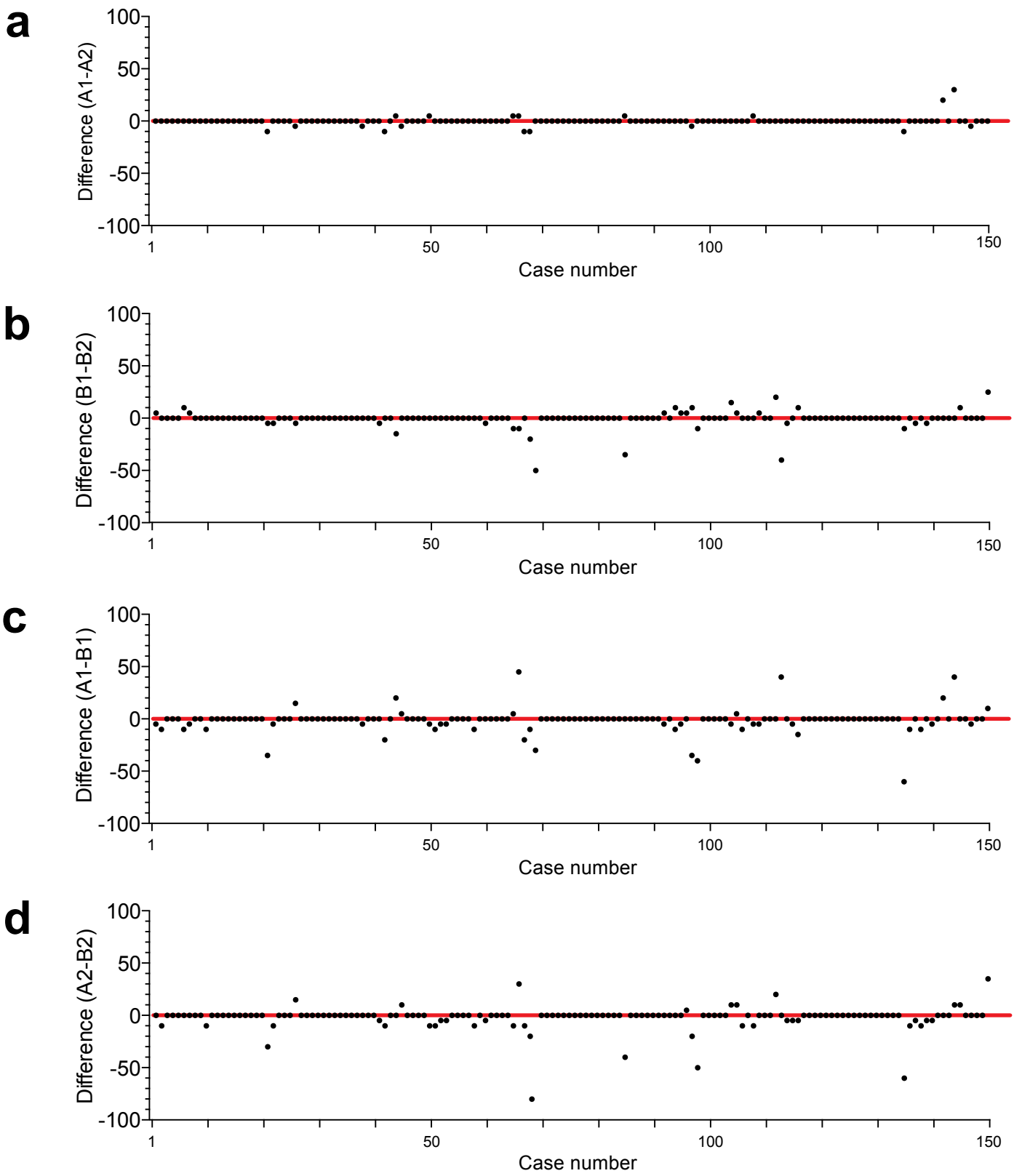
Supplementary Figure 15



Supplementary Figure 15 Knockdown of ARPC3 does not effect tumor burden or tumor vessel density in mice treated with capecitabine alone

a-c. Tumors with normal ARPC3 levels (Control shRNA) or ARPC3 knockdown (shARPC3-3) were established in the livers of mice. Mice were then treated with capecitabine (C) or vehicle alone (Vh) for two weeks followed by histopathological analysis of the liver tumors (n = 8 mice per group). Graph in **a** shows the % HGP per group ± SEM. Graph in **b** shows liver tumor burden expressed in terms of lesion area ± SEM. Graph in **c** shows tumor vessel density in terms of vessels per mm^2 ± SEM. For statistical analysis, Mann Whitney U-test (panel **a**) or Student's t-test (panels **b,c**) were used. ***P*<0.01. n.s., no significant difference.

Supplementary Figure 16



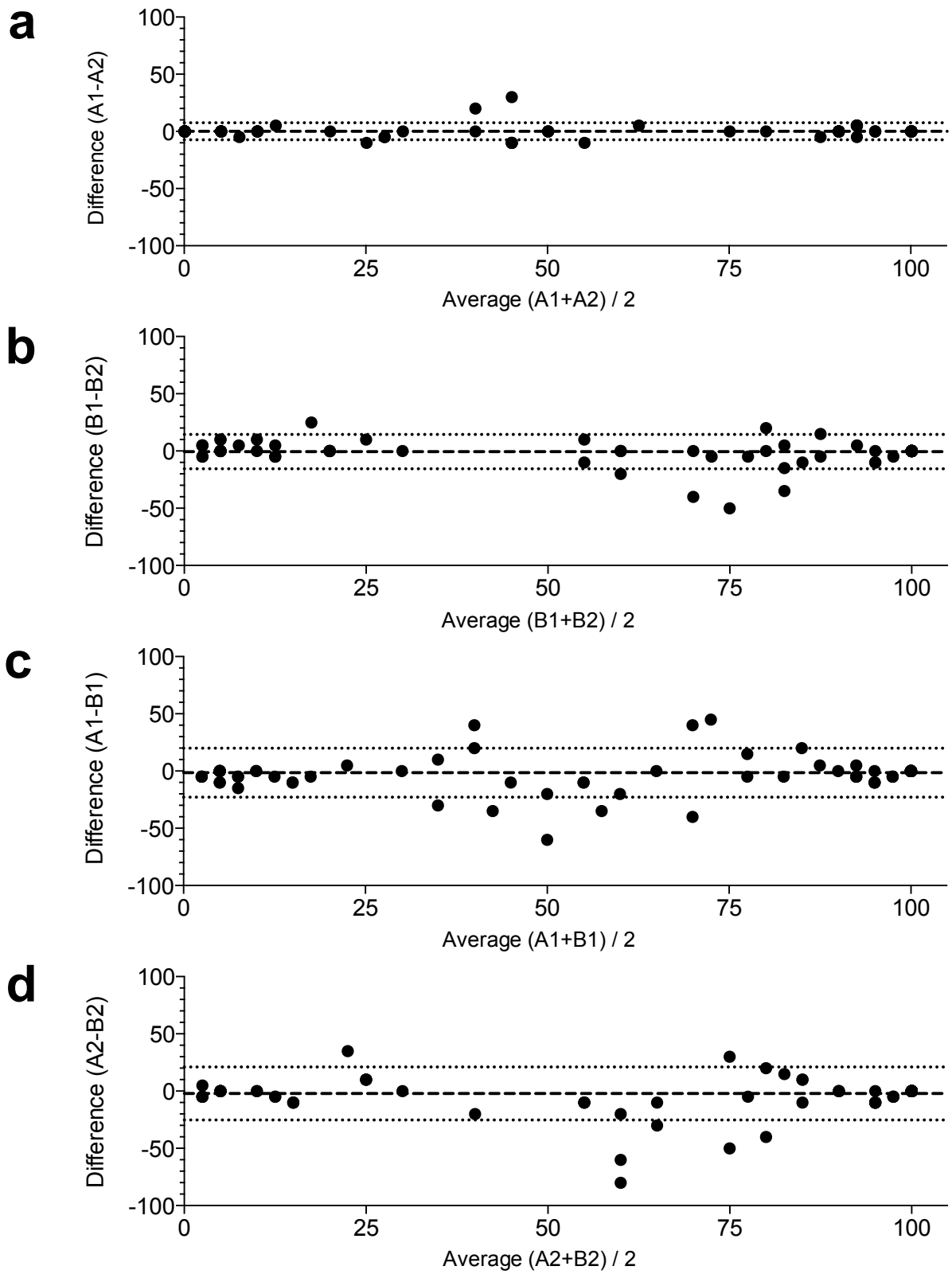
Supplementary Figure 16 Difference in % HGP scores between observers for the intra-observer and inter-observer agreement of HGP scoring

Two observers scored the HGP (% replacement, % desmoplastic, % pushing) in 150 tissue sections of colorectal cancer liver metastasis. The graphs show the difference between the two % replacement scores for every case for the following comparisons:

a. intra-observer agreement: observer A first score (A1) minus observer A second score (A2), **b.** intra-observer agreement: observer B first score (B1) minus observer B second score (B2), **c.** inter-observer agreement: observer A first score (A1) minus observer B first score (B1) and **d.** inter-observer agreement: observer A second score (A2) minus observer B second score (B2).

Data points which lie on the red line indicate cases for which there was complete agreement between the two scores, whilst data points either side of the line are cases for which there was disagreement between the two scores.

Supplementary Figure 17



Supplementary Figure 17 Bland-Altman plots for intra-observer and inter-observer agreement of HGP scoring
Two observers scored the HGP (% replacement, % desmoplastic, % pushing) in 150 tissue sections of colorectal cancer liver metastasis. Bland-Altman plots show the difference between the two % replacement scores plotted against the average of the two % replacement scores for the following comparisons:
a. Intra-observer agreement: observer A first score (A1) versus observer A second score (A2). Mean difference between scores (-0.033) and limits of agreement (-7.431 to 7.497). **b.** Intra-observer agreement: observer B first score (B1) versus observer B second score (B2). Mean difference between scores (-0.633) and limits of agreement (-15.663 to 14.397). **c.** Inter-observer agreement: observer A first score (A1) versus observer B first score (B1). Mean difference between scores (-1.500) and limits of agreement (-22.88 to 19.88). **d.** Inter-observer agreement: observer A second score (A2) versus observer B second score (B2). Mean difference between scores (-2.167) and limits of agreement (-25.287 to 20.953).
Bold dashed line indicates the mean difference between scores whilst the flanking dotted lines show the limits of agreement. Note: since a large proportion of the 150 data points in each graph have identical x and y co-ordinates, many of the data points depicted constitute multiple overlaping data points.

Supplementary Table 1 Characteristics of bev–chemo treated CRC patients in the RM cohort

Characteristics of 33 patients (n = 59 lesions) treated preoperatively with bev-chemo prior to liver resection at RM.

Demographics	
Gender, number of patients (%)	
Male	21 (63.6)
Female	12 (36.4)
Age, median (range)	63 (29 – 79)
Primary tumor	
Site of primary tumor, number of patients (%)	
Rectum	7 (21.2)
Recto–sigmoid	14 (42.4)
Colon	12 (36.4)
Lymph node status, number of patients (%)	
Positive	26 (78.8)
Negative	7 (21.2)
Histological grade, number of patients (%)	
High grade	4 (12.1)
Low grade	29 (87.9)
Adjuvant therapy, number of patients (%)	
Yes	10 (30.3)
No	23 (69.7)
Liver metastasis	
No. of liver lesions at presentation, number of patients (%)	
Solitary lesion	11 (33.3)
Multiple lesions	22 (66.7)
No. of liver lesions utilised for histopathological analysis per patient, number of patients (%)	
1 lesion	17 (51.5)
2 lesions	10 (30.3)
3 lesions	2 (6.1)
4 lesions	4 (12.1)
Baseline lesion size, median (range)	21 mm (5 – 110)
Preoperative therapy administered, number of patients (%)	
CAPOX + bevacizumab	21 (63.6)
FOLFOX + bevacizumab	5 (15.2)
FOLFIRI + bevacizumab	7 (21.2)
Cycles of preoperative therapy, median (range)	6 (4 – 12)
Interval between last bevacizumab dose and resection, median (range)	76 days (41 – 362)

Footnote: CAPOX, capecitabine and oxaliplatin; FOLFOX, infusional 5–fluorouracil and oxaliplatin; FOLFIRI, infusional 5–fluorouracil and irinotecan.

Supplementary Table 2 Univariate analysis of clinical characteristics associated with pathological response in RM patients treated preoperatively with bev–chemo

Analysis was performed using data for 59 lesions from 33 patients treated preoperatively with bev-chemo prior to liver resection (RM cohort). The χ^2 test was used to determine statistical significance.

Variables	Total number of lesions	Lesions with <25% viable tumor, no. (%)	*P*–value
Demographics			
Gender			
Male	34	12 (35.3)	0.712
Female	25	10 (40)	
Age			
<60 years	17	6 (35.3)	0.840
≥60 years	42	16 (38.1)	
Primary tumor			
Site of primary tumor			
Rectum	13	4 (30.8)	0.599
Recto–sigmoid	24	8 (33.3)	
Colon	22	10 (45.5)	
Lymph node status			
Positive	48	19 (39.6)	0.446
Negative	11	3 (27.3)	
Histological grade			
High grade	8	5 (62.5)	0.113
Low grade	51	17 (33.3)	
Adjuvant therapy			
Yes	18	4 (22.2)	0.113
No	41	18 (43.9)	
Liver metastasis			
No. of liver lesions at presentation			
Solitary	11	5 (45.5)	0.535
Multiple	48	17 (35.4)	
Baseline lesion size			
<20 mm	24	11 (45.8)	0.261
≥20 mm	35	11 (31.4)	
Preoperative therapy administered			
CAPOX + bevacizumab	37	16 (42.1)	0.475
FOLFOX + bevacizumab	9	2 (22.2)	
FOLFIRI + bevacizumab	13	4 (30.8)	
Cycles of preoperative therapy			
≤6 cycles	44	16 (36.4)	0.801
>6 cycles	15	6 (40.0)	
Interval between last bevacizumab dose and resection			
<70 days	24	10 (41.7)	0.565
≥70 days	35	12 (34.3)	

Table continues overleaf

Supplementary Table 2 continued

Variables	Total number of lesions	Lesions with <25% viable tumor, no (%)	*P*–value
Response measures			
Change in lesion size by RECIST			
PR	34	15 (44.1)	0.206
SD or PD	25	7 (28.0)	
Morphological response on CT			
Yes (OR or PR)	19	11 (57.9)	0.051
No (AR)	33	10 (30.3)	
Histopathological growth pattern			
Replacement HGP			
<25%	28	20 (71.4)	<0.001
≥25%	31	2 (6.5)	
Replacement HGP			
<50%	32	21 (65.6)	<0.001
≥50%	27	1 (3.7)	
Desmoplastic HGP			
<25%	25	0 (0)	<0.001
≥25%	34	22 (64.7)	
Desmoplastic HGP			
<50%	28	1 (3.6)	<0.001
≥50%	31	21 (67.7)	

Footnote: CAPOX, capecitabine and oxaliplatin; FOLFOX, infusional 5–fluorouracil and oxaliplatin; FOLFIRI, infusional 5–fluorouracil and irinotecan; N/A, data not available.

Supplementary Table 3 Characteristics of bev–chemo treated CRC patients in the MUHC cohort

Characteristics of 59 patients (n = 128 lesions) treated preoperatively with bev-chemo at MUHC.

Demographics	
Gender, number of patients (%)	
Male	35 (59.3)
Female	24 (40.7)
Age, median (range)	63 (30 – 85)
Primary tumor	
Site of primary tumor, number of patients (%)	
Rectum	11 (18.6)
Recto–sigmoid	9 (15.3)
Colon	39 (66.1)
Lymph node status, number of patients (%)	
Positive	32 (54.2)
Negative	8 (13.6)
N/A	19 (32.2)
Histological grade, number of patients (%)	
High grade	4 (6.8)
Low grade	36 (61.0)
N/A	19 (32.2)
Adjuvant therapy, number of patients (%)	
Yes	12 (20.3)
No	46 (78.0)
N/A	1 (1.7)
Liver metastasis	
No. of liver lesions at presentation, number of patients (%)	
Solitary lesion	18 (30.5)
Multiple lesions	41 (69.5)
No. of liver lesions utilised for histopathological analysis per patient, number of patients (%)	
1 lesion	29 (49.2)
2 lesions	15 (25.4)
3 lesions	7 (11.8)
4 lesions	3 (5.1)
5 lesions	2 (3.4)
6 lesions	1 (1.7)
8 lesions	1 (1.7)
12 lesions	1 (1.7)
Baseline lesion size, median (range)	26 (5 – 190)*
Preoperative therapy administered, number of patients (%)	
FOLFOX + bevacizumab	47 (79.7)
FOLFIRI + bevacizumab	12 (20.3)
Cycles of preoperative therapy, median (range)	6 (2 – 13)
Interval between last bevacizumab dose and resection, median (range)	64 (23 – 237)

Footnote: FOLFOX, infusional 5–fluorouracil and oxaliplatin; FOLFIRI, infusional 5–fluorouracil and irinotecan; N/A, data not available. *Information on baseline lesion size was available for 113 out of 128 lesions.

Supplementary Table 4 Univariate analysis of clinical characteristics associated with pathological response in MUHC patients treated preoperatively with bev–chemo

Analysis was performed using data for 128 lesions from 59 patients treated preoperatively with bev-chemo prior to liver resection (MUHC cohort). The χ^2 test was used to determine statistical significance.

Variables	Total number of lesions	Lesions with <25% viable tumor, no. (%)	*P*–value
Demographic			
Gender			
Male	88	29 (32.9)	0.297
Female	40	17 (42.5)	
Age			
<60 years	53	18 (34.0)	0.695
≥60 years	75	28 (37.3)	
Primary tumor			
Site of primary tumor			
Rectum	21	5 (23.8)	0.022
Recto–sigmoid	14	8 (57.1)	
Colon	93	33 (35.5)	
Lymph node status			
Positive	66	20 (30.3)	0.032
Negative	11	7 (63.6)	
Histological grade			
High grade	6	1 (16.7)	0.279
Low grade	72	28 (38.9)	
Adjuvant therapy			
Yes	24	6 (25)	0.204
No	103	40 (38.8)	
Liver metastasis			
No. of liver lesions at presentation			
Solitary	18	7 (38.9)	0.778
Multiple	110	39 (35.4)	
Baseline lesion size			
<20 mm	40	13 (32.5)	0.447
≥20 mm	73	29 (39.7)	
Preoperative therapy administered			
FOLFOX + bevacizumab	108	42 (38.9)	0.048
FOLFIRI + bevacizumab	20	4 (20.0)	
Cycles of preoperative therapy			
≤6 cycles	86	37 (43)	0.017
>6 cycles	42	9 (21.4)	
Interval between last bevacizumab dose and resection			
<70 days	58	22 (37.9)	0.669
≥70 days	70	24 (34.3)	

Table continues overleaf

Supplementary Table 4 continued

Variables	Total number of lesions	Lesions with <25% viable tumor, no (%)	*P*–value
Response measures			
Change in lesion size by RECIST			
PR	44	22 (50)	0.024
SD or PD	69	20 (29)	
Histopathological growth pattern			
Replacement HGP			
<25%	60	34 (56.7)	<0.001
≥25%	68	23 (17.7)	
Replacement HGP			
<50%	70	40 (57.1)	<0.001
≥50%	58	6 (10.3)	
Desmoplastic HGP			
<25%	48	2 (4.2)	<0.001
≥25%	80	44 (55)	
Desmoplastic HGP			
<50%	62	6 (9.7)	<0.001
≥50%	66	40 (60.6)	

Footnote: FOLFOX, infusional 5–fluorouracil and oxaliplatin; FOLFIRI, infusional 5–fluorouracil and irinotecan; N/A, data not available.

Supplementary Table 5 Univariate and multivariate analysis of clinical characteristics associated with pathological response in lesions treated preoperatively with bev–chemo

Data from patients that received preoperative therapy with bev–chemo were used to determine clinical variables associated with a good pathological response (lesions were pooled from RM and MUHC). Only lesions with ≥50% replacement HGP (85 lesions) or ≥50% desmoplastic HGP (96 lesions) were included. Lesions with ≥50% pushing HGP were excluded (6 lesions). The final analysis was therefore performed on 181 lesions from 90 patients. Both the univariate analysis and the multivariate analysis were performed using a generalized estimating equation. Only 5 variables that met a pre–defined threshold of $P<0.25$ in the univariate analysis were included in the subsequent multivariate analysis.

	Univariate analysis		Multivariate analysis	
	OR (95% CI)	***P*–value**	**OR (95% CI)**	***P*–value**
Demographics				
Gender				
Male	0.83 (0.69 – 1.00)	0.0507	0.80 (0.32 – 2.00)	0.6304
Female	1.21 (1.00 – 1.45)		1.25 (0.50 – 3.16)	
Age			–	–
<60 years	1.03 (0.85 – 1.24)	0.7629		
≥60 years	0.97 (0.81 – 1.18)			
Primary tumour				
Site of primary tumor				
Rectum	0.91 (0.74 – 1.11)	0.3502	–	–
Colon / recto–sigmoid	1.10 (0.90 – 1.35)			
Lymph node status				
Positive	0.68 (0.25 – 1.89)	0.4565	–	–
Negative	1.47 (0.53 – 4.06)			
Histological grade				
High grade	1.16 (0.30 – 4.55)	0.8259	–	–
Low grade	0.86 (0.22 – 3.35)			
Adjuvant therapy				
Yes	0.85 (0.70 – 1.03)	0.1087	0.48 (0.17 – 1.41)	0.1834
No	1.17 (0.97 – 1.42)		2.07 (0.71 – 6.01)	
Liver metastasis				
Number of lesions at presentation				
Solitary	1.07 (0.87 – 1.32)	0.5275	–	–
Multiple	0.93 (0.76 – 1.15)			
Baseline lesion size				
<20 mm	0.99 (0.49 – 2.01)	0.9730	–	–
≥20 mm	1.01 (0.50 – 2.04)			
Preoperative therapy administered				
CAPOX + bev / FOLFOX + bev	2.09 (0.76 – 5.78)	0.1534	1.14 (0.37 – 3.51)	0.8237
FOLFIRI + bev	0.48 (0.17 – 1.32)		0.88 (0.29 – 2.70)	
Cycles of preoperative therapy				
≤6 cycles	2.03 (0.82 – 5.02)	0.1249	1.74 (0.71 – 4.28)	0.2256
>6 cycles	0.49 (0.20 – 1.22)		0.57 (0.23 – 1.41)	
Interval between last bevacizumab dose and resection				
<70 days	1.41 (0.66 – 3.03)	0.3782	–	–
≥70 days	0.71 (0.33 – 1.52)			
HGP				
≥50% replacement	0.07 (0.03 – 0.16)	<0.0001	0.06 (0.03 – 0.15)	<0.0001
≥50% desmoplastic	15.06 (6.32 – 35.87)		15.92 (6.76 – 37.51)	

Footnote: For every variable tested, we present the odds ratio in both directions e.g. male vs female (OR=0.83) and its reverse, female vs male (OR=1.21), etc.

bev, bevacizumab; CAPOX, capecitabine and oxaliplatin; FOLFOX, infusional 5–fluorouracil and oxaliplatin; FOLFIRI, infusional 5–fluorouracil and irinotecan.

Supplementary Table 6 Characteristics of MUHC patients that presented with new CRC liver metastases after bev–chemo treatment was initiated (new CRCLMs)

Demographics	
Gender, number of patients (%)	
Male	9 (69.2)
Female	4 (30.8)
Age, median (range)	65 (46–78)
Primary tumor	
Site of primary tumor, number of patients (%)	
Rectum	2 (15.4)
Recto–sigmoid	3 (23.1)
Colon	8 (61.5)
Lymph node status, number of patients (%)	
Positive	10 (76.9)
Negative	0
N/A	3 (23.1)
Histological grade, number of patients (%)	
High grade	2 (15.4)
Low grade	8 (61.5)
N/A	3 (23.1)
Adjuvant therapy, number of patients (%)	
Yes	4 (30.8)
No	9 (69.2)
Liver metastasis	
Quantity of liver lesions present when treatment started, number of patients (%)	
No lesion*	2 (15.4)
Solitary lesion	2 (15.4)
Multiple lesions	9 (69.2)
Quantity of new liver lesions presenting after treatment started, number of patients (%)	
Solitary lesion	7 (53.8)
Multiple lesions	6 (46.2)
No. of liver lesions utilised for histopathological analysis per patient, number of patients (%)	
1 lesion	7 (53.8)
2 lesions	3 (23.1)
3 lesions	1(7.7)
5 lesions	1 (7.7)
14 lesions	1 (7.7)
Preoperative therapy administered, number of patients (%)	
FOLFOX + bevacizumab	9 (69.2)
FOLFIRI + bevacizumab	4 (30.8)
Cycles of preoperative therapy, median (range)	6 (5 – 12)
Interval between last bevacizumab dose and resection, median (range)	67 days (43 – 126)

Footnote: *Two patients were administered bev-chemo prior to detection of liver metastases: one patient was receiving adjuvant bev-chemo when liver disease was detected and a second patient was receiving bev-chemo for CRC lung metastasis when liver disease was detected. bev, bevacizumab; CAPOX, capecitabine and oxaliplatin; FOLFOX, infusional 5–fluorouracil and oxaliplatin; FOLFIRI, infusional 5–fluorouracil and irinotecan. N/A, data not available.

Supplementary Table 7 Characteristics of MUHC patients that received no preoperative therapy prior to resection of CRC liver metastases (untreated CRCLMs)

Demographics	
Gender, number of patients (%)	
Male	11 (57.9)
Female	8 (42.1)
Age, median (range)	70 (33 – 80)
Primary tumor	
Site of primary tumor, number of patients (%)	
Rectum	5 (26.3)
Recto–sigmoid	1 (5.3)
Colon	13 (68.4)
Lymph node status, number of patients (%)	
Positive	10 (52.6)
Negative	5 (26.3)
N/A	4 (21.1)
Histological grade, number of patients (%)	
High grade	1 (5.3)
Low grade	10 (52.6)
N/A	8 (42.1)
Adjuvant therapy, number of patients (%)	
Yes*	4 (21.1)
No (completely chemonaive)	15 (78.9)
Baseline features of the liver metastases	
No. of liver lesions at presentation, number of patients (%)	
Solitary lesion	12 (63.2)
Multiple lesions	7 (36.8)
No. of liver lesions utilised for histopathological analysis per patient, number of patients (%)	
1 lesion	12 (61.1)
2 lesions	5 (26.3)
4 lesions	1 (5.3)
6 lesions	1 (5.3)
Baseline lesion size, median (range)	13.5 mm (4 – 77)

Footnote: *patients were only included if the last dose of adjuvant therapy was administered ≥ 365 days prior to diagnosis of liver metastasis (median interval between last dose of adjuvant therapy and diagnosis of liver metastasis in these 4 patients was 1161 days, range was 789 – 1667 days). Adjuvant therapy consisted of chemotherapy only and no patients received adjuvant bevacizumab. N/A, data not available.

Supplementary Table 8 Univariate and multivariate analysis of clinical characteristics associated with overall survival in patients treated preoperatively with bev–chemo

Data from patients that received preoperative therapy with bev–chemo at MUHC were used to determine clinical variables associated with overall survival. Only patients in the predominant replacement subgroup (26 patients) or the predominant desmoplastic subgroup (35 patients) were included in the analysis. The predominant pushing subgroup (1 patient) was excluded from the analysis. The final analysis was therefore performed on 61 patients. Both the univariate analysis and the multivariate analysis were performed using Cox proportional hazards regression. Only 2 variables that met a pre–defined threshold of $P<0.25$ in the univariate analysis were included in the subsequent multivariate analysis.

	Univariate analysis		Multivariate analysis	
	HR (95% CI)	***P*–value**	**HR (95% CI)**	***P*–value**
Demographics				
Gender				
Male	1.14 (0.49 – 2.63)	0.7641	–	–
Female	0.88 (0.38 – 2.06)			
Age				
<60 years	1.08 (0.47 – 2.48)	0.8494	–	–
≥60 years	0.93 (0.40 – 2.13)			
Primary tumour				
Site of primary tumor				
Rectum	1.28 (0.43 – 3.78)	0.6504	–	–
Colon / recto–sigmoid	0.78 (0.26 – 2.33)			
Lymph node status				
Positive	0.72 (0.16 – 3.23)	0.6788	–	–
Negative	1.38 (0.31 – 6.21)			
Histological grade				
High grade	1.25 (0.35 – 4.35)	0.7324	–	–
Low grade	0.80 (0.23 – 2.83)			
Adjuvant therapy				
Yes	1.05 (0.35 – 3.13)	0.9274	–	–
No	0.95 (0.32 – 2.86)			
Liver metastasis				
Number of lesions at presentation				
Solitary	0.41 (0.15 – 1.11)	0.0797	0.51 (0.19 – 1.42)	0.1985
Multiple	2.44 (0.90 – 6.67)		1.96 (0.70 – 5.26)	
Mean baseline lesion size				
<20 mm	1.63 (0.65 – 4.06)	0.2957	–	–
≥20 mm	0.61 (0.25 – 1.54)			
Preoperative therapy administered				
CAPOX+bev / FOLFOX+bev	0.91 (0.36 – 2.31)	0.8476	–	–
FOLFIRI+bev	1.10 (0.43 – 2.78)			
Cycles of preoperative therapy				
≤6 cycles	0.67 (0.30 – 1.51)	0.3315	–	–
>6 cycles	1.49 (0.66 – 3.33)			
Interval between last bevacizumab dose and resection				
<70 days	1.03 (0.44 – 2.38)	0.9488	–	–
≥70 days	0.97 (0.42 – 2.27)			
HGP				
≥50% replacement	0.29 (0.12 – 0.67)	0.0040	0.33 (0.14 – 0.80)	0.0135
≥50% desmoplastic	3.50 (1.49 – 8.20)		3.03 (1.25 – 7.14)	

Footnote: For each variable tested, we present the odds ratio in both directions e.g. male vs female (HR=1.14) and its reverse, female vs male (HR=0.88), etc.

bev, bevacizumab; CAPOX, capecitabine and oxaliplatin; FOLFOX, infusional 5–fluorouracil and oxaliplatin; FOLFIRI, infusional 5–fluorouracil and irinotecan.

Supplementary Table 9 Analysis for differences in characteristics between patients with a predominant replacement HGP and patients with a predominant desmoplastic HGP

Analysis was performed on 89 patients from MUHC that received preoperative therapy with bev–chemo or chemotherapy alone. Clinical characteristics were compared between 38 predominant replacement HGP patients and 51 predominant desmoplastic HGP patients. The χ^2 test was used to determine statistical significance.

	Total number of patients	Number of replacement patients (%)	Number of desmoplastic patients (%)	*P*–value
Demographics				
Gender				
Male	56	28 (50)	28 (50)	0.070
Female	33	10 (30.3)	23 (69.7)	
Age				
<60 years	35	15 (42.9)	20 (57.1)	0.980
≥60 years	54	23 (42.6)	31 (57.4)	
Primary tumour				
Primary tumour site				
Rectum	20	7 (35)	13 (65)	0.544
Recto–sigmoid	17	9 (52.9)	8 (47.1)	
Colon	32	22 (68.8)	10 (31.2)	
Lymph nodes				
Positive	44	20 (45.5)	24 (54.5)	0.522
Negative	14	5 (35.7)	9 (64.3)	
Histological grade				
High grade	6	4 (66.7)	2 (33.3)	0.149
Low grade	55	20 (36.4)	35 (63.6)	
Treated with adjuvant therapy				
Yes	16	8 (50)	8 (50)	0.543
No	72	30 (41.7)	42 (58.3)	
Liver metastasis				
Number of lesions at presentation				
No lesion*	3	3 (100)	0 (0)	0.046
Solitary lesion	27	8 (29.6)	19 (70.4)	
Multiple lesions	59	27 (45.8)	32 (54.2)	
Mean baseline lesion size				
<20 mm	25	9 (36)	16 (64)	0.666
≥20 mm	56	23 (41.1)	33 (58.9)	
Therapy administered				
FOLFOX	24	11 (45.8)	13 (54.2)	0.679
FOLFIRI	1	0 (0)	1 (100)	
FOLFIRINOX	2	1 (50)	1 (50)	
5–FU	1	0	1 (100)	
FOLFOX + bev	49	19 (38.8)	30 (61.2)	
FOLFIRI + bev	12	7 (58.3)	5 (41.7)	

Table continues overleaf

Supplementary Table 9 continued

Cycles of preoperative therapy				
≤6 cycles	62	26 (41.9)	36 (58.1)	0.826
>6 cycles	27	12 (44.4)	15 (55.6)	
Interval between last therapy dose and resection				
<70 days	47	15 (31.9)	32 (68.1)	0.030
≥70 days	38	21 (55.3)	17 (44.7)	

Footnote: *Three patients were administered therapy prior to detection of liver metastases: one patient was receiving adjuvant bev-chemo when liver disease was detected, one patient was receiving bev-chemo for CRC lung metastasis when liver disease was detected and one patient was receiving adjuvant chemotherapy alone when liver disease was detected. FOLFOX, infusional 5–fluorouracil and oxaliplatin; FOLFIRI, infusional 5–fluorouracil and irinotecan; FOLFIRINOX, infusional 5–fluorouracil and irinotecan and oxaliplatin; 5–FU, infusional 5–FU only.

Supplementary Table 10 Analysis for differences in characteristics between patients that received bev–chemo and patients that received chemotherapy alone

Analysis was performed on 91 patients from MUHC. Clinical characteristics were compared between 62 patients that received pre–operative bev–chemo and 29 patients that received preoperative chemotherapy only). The χ^2 test was used to determine statistical significance.

	Total number of patients	Number of bev–chemo patients (%)	Number of chemo alone patients (%)	*P*–value
Demographics				
Gender				
Male	57	37 (64.9)	20 (35.1)	0.393
Female	34	25 (73.5)	9 (26.5)	
Age				
<60 years	36	25 (69.4)	11 (30.6)	0.828
≥60 years	55	37 (67.3)	18 (32.7)	
Primary tumor				
Primary tumour site				
Rectum	21	12 (57.1)	9 (42.9)	0.206
Recto–sigmoid	17	10 (58.8)	7 (41.2)	
Colon	53	40 (75.5)	13 (24.5)	
Lymph nodes				
Positive	45	35 (77.8)	10 (22.2)	0.129
Negative	14	8 (57.1)	6 (42.9)	
Histological grade				
High grade	6	5 (83.3)	1 (16.7)	0.468
Low grade	55	38 (69.1)	17 (30.9)	
Treated with adjuvant therapy				
Yes	18	13 (72.2)	5 (27.8)	0.652
No	72	48 (66.7)	24 (33.3)	
Liver metastases				
Number of lesions at presentation				
No lesion*	4	2 (50)	2 (50)	0.695
Solitary lesion	27	18 (66.7)	9 (33.3)	
Multiple lesions	60	42 (70)	18 (30)	
Mean baseline lesion size				
<20 mm	25	14 (56)	11 (44)	0.125
≥20 mm	56	41 (73.2)	15 (26.8)	
Therapy administered				
FOLFOX	75	50 (66.7)	25 (33.3)	0.019
FOLFIRI	13	12 (92.3)	1 (7.7)	
FOLFIRINOX	2	0 (0)	2 (100)	
5–FU	1	0 (0)	1 (100)	

Table continues overleaf

Supplementary Table 10 continued

Cycles of preoperative therapy				
≤6 cycles	63	41 (65.1)	22 (34.9)	0.349
>6 cycles	28	21 (75)	7 (25)	
Interval between last therapy dose & resection				
<70 days	48	35 (72.9)	13 (27.1)	0.527
≥70 days	39	26 (66.7)	13 (33.3)	

Footnote: *Four patients were administered therapy prior to detection of liver metastases: one patient was receiving adjuvant bev–chemo when liver disease was detected, one patient was receiving bev–chemo for CRC lung metastasis when liver disease was detected and two patients were receiving adjuvant chemotherapy alone when liver disease was detected. FOLFOX, infusional 5–fluorouracil and oxaliplatin; FOLFIRI, infusional 5–fluorouracil and irinotecan; FOLFIRINOX, infusional 5–fluorouracil and irinotecan and oxaliplatin; infusional 5–FU.

Supplementary Table 11 Characteristics of 17 patients from whom samples of breast cancer liver metastasis were obtained

Details of primary	
Age at diagnosis of primary breast cancer, median (range)	47 (36 – 77)
Primary was resected, number of patients (%)	
Yes	15 (88.2)
No	2 (11.8)
Ductal or lobular histology, number of patients (%)	
Ductal	13 (76.5)
Lobular	3 (17.6)
Mixed	1 (5.9)
T–stage, number of patients (%)	
T1	6 (35.3)
T2	6 (35.3)
T3	2 (11.8)
T4	1 (5.9)
N/A	2 (11.8)
Lymph nodes, number of patients (%)	
Positive	9 (52.9)
Negative	6 (35.3)
N/A	2 (11.8)
Treatment received prior to obtaining liver metastasis sample	
Form of treatment received, number of patients (%)	
Endocrine therapy	14 (82.4)
Chemotherapy	12 (70.6)
Herceptin	2 (11.8)
Everolimus	1 (5.9)
Iressa	1 (5.9)
Zometa	1 (5.9)
Details of liver metastasis sample	
Age when sample was obtained, median (range)	54 (43 – 81)
Source of material, number of patients (%)	
Resection	11 (64.7)
Autopsy	6 (35.3)
Intrinsic subtype, number of patients (%)	
Luminal A	5 (29.4)
Luminal B HER2 negative	5 (29.4)
Luminal B HER2 positive	3 (17.7)
HER2 positive (non–luminal)	0 (0)
Triple negative	4 (23.5)

Footnote: N/A, data not available.

Supplementary Table 12 Results of the intra– and inter–observer agreement study for scoring the HGPs of liver metastases

Measurement of intra–observer agreement for HGP scoring			
Comparison	**Correlation co–efficient**	**Mean difference**	**Limits of agreement**
Observer A (1st score) versus Observer A (2nd score)	0.9965	0.033	(–7.431 to 7.497)
Observer B (1st score) versus Observer B (2nd score)	0.9866	–0.633	(–15.663 to 14.397)

Measurement of inter–observer agreement for HGP scoring			
Comparison	**Correlation co–efficient**	**Mean difference**	**Limits of agreement**
Observer A (1st score) versus Observer B (1st score)	0.9715	–1.500	(–22.88 to 19.88)
Observer A (2nd score) versus Observer B (2nd score)	0.9678	–2.167	(–25.287 to 20.953)

Supplementary Table 13 Criteria for scoring the intrinsic subtypes of breast cancer

Intrinsic subtype	Criteria
Luminal A	ER and PgR positive HER2 negative Ki67 ‘low’
Luminal B HER2–negative	ER positive HER2 negative Ki67 ‘high’
Luminal B HER2–positive	ER positive HER2 positive Any Ki67 Any PgR
HER2 positive (non–luminal)	HER2 positive ER and PgR absent
Triple negative	ER negative PgR negative HER2 negative

Footnote: Table was adapted from: Goldhirsch, A.*, et al.* Personalizing the treatment of women with early breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013. *Ann Oncol* **24**, 2206–2223 (2013). ER, estrogen receptor; PgR, progesterone receptor.